Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
FEB 2009		**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Safety Evaluation of Silver Nanoparticles: Inhalation Model for Chronic Exposure	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Air Force Research Laboratory,711th Human Performance Wing,Applied Biotechnology Branch, Human Effectiveness Directorate,Wright-Patterson AFB,OH,45433**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**2**	

TOXICOLOGICAL SCIENCES **108(2)**, 223–224 (2009)
doi:10.1093/toxsci/kfp032
Advance Access publication February 23, 2009

TOXICOLOGICAL HIGHLIGHT

Safety Evaluation of Silver Nanoparticles: Inhalation Model for Chronic Exposure

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Received February 9, 2009; accepted February 10, 2009

The rapid emergence of nanotechnology including production of engineered nanoparticles has provided many exciting advancements in science and is likely to provide our society with a continuous range of consumer products with advanced technology applications. For example, use of the inexhaustible propensity of inherent antimicrobial activity for silver nanoparticles has resulted in their widespread application and use in consumer products such as disinfectants, deodorants, antimicrobial sprays and powders, bedding, machine washers, humidifiers, water purification and air filters, toothpaste, shampoo and rinse, reusable bottle nursing nipples, in multiple fabrics, kitchen utensils, and toys. Among the 580 consumer products containing known nanomaterials, the most common material mentioned in product descriptions is silver-based nanoparticles (Woodrow Wilson International Center for Scholars, 2007). Human exposure likelihood and needed risk exposure analysis to these product-derived nanomaterials are now required for every age level. Further, disposal and degradation of these products and release from engineered sources has an indirect human exposure potential and an environmental impact concern.

The approach to assess toxicity of nanomaterials is a relatively new and evolving field. Most nanotoxicology studies have focused on mechanistic understanding using *in vitro* models with the early reports demonstrating that high levels of silver nanoparticles are lethal to eukaryotic cell–based systems (Braydich-Stolle *et al.*, 2005; Carlson et al., 2008; Hussain *et al.*, 2005, 2006). Cytotoxicity occurs through the generation of radical oxygen species (Hussain *et al.*, 2005), and more recently, silver nanoparticles have been shown to cause DNA damage in mammalian cells (Ahamed *et al.*, 2008).

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Nanotoxicology assessment studies performed *in vivo* using appropriate dosing amounts and routes of exposure carry greater significance because of the diversity of systemic phenotypic response and the physiologic/anatomic influence that can be translatable from animal models to human exposures. Particle pharmacokinetics and dynamics of nondissolved solution materials combined with issues of regional dissolution rates can provide the true impact of dosing on systemic physiology and positional anatomy. The present information on silver nanoparticle toxicity *in vivo* has been limited. In a 28-day oral study, Kim *et al.* (2008) reported clinical chemistry and hematology along with providing histopathological examination to evaluate the distribution of silver in rats. Male and female rats did not show any significant changes in body weight. However, significant dose-dependent changes were found for serum alkaline phosphatase and cholesterol values in both male and female rats, which provided an indication that high-level oral exposure (300 μg silver nanoparticles) may result in moderate liver damage. A micronucleus analysis showed that there were no statistically significant differences in the micronucleated polychromatic erythrocytes totals after silver nanoparticle exposure when compared to the control. These authors did not find toxicity in male and female rat bone marrow. However, a gender-related difference for the accumulation of silver was noted in kidneys with a twofold higher concentration in female kidneys when compared with the male kidneys.

Based on the above studies, Sung *et al.* (2008) in their work presented in this issue reports on a Sprague-Dawley rat whole body exposure study for defining the subchronic 90-day inhalation toxicity of silver nanoparticles. The authors made an important finding in this study. This subchronic 90-day silver studies on nanoparticle inhalation indicated that the lungs and liver were the major target tissues for prolonged silver nanoparticle exposure. The higher accumulation of silver nanoparticles in the female kidneys was again found, and higher concentrations were determined to be located in the kidney basement membranes of the medulla and cortex. The exact

functional toxicity consequence is not apparent for the gender difference with this higher concentration silver nanoparticle since no significant kidney function or histopathologic changes were found in the 90-day time course for the female kidneys. This present inhalation study suggests accumulation of silver nanoparticles in the female kidneys was not dependent on route of administration as the same finding was discovered in oral studies (Kim et al., 2008). Lastly, whether alpha-2-micro-globulin found only in male rats contributes in this gender-dependent uptake needs further assessment. Direct lung effects have been previously reported as causing a decrease in tidal and minute volume and eliciting inflammatory responses, such as a mixed inflammatory cell infiltrate and chronic alveolar inflammation (Sung *et al*., 2008). Furthermore, although on a minimal level, silver nanoparticle exposure-related bile duct hyperplasia was noted in both the male and female animals. Similar histopathological observations for the 28-day oral dose study showed a dose-dependent increased incidence of bile duct hyperplasia around the central vein to the hepatic lobule with the infiltration of inflammatory cells, including eosinophils (Kim *et al*., 2008).

As previously observed in the 28-day inhalation and 28-day oral dose studies (Kim *et al*., 2008), silver nanoparticles were distributed in all the tissues examined in this investigation. However, when compared with the results of the oral dose study, this study found that the upper nasal deposition caused olfactory bulb accumulation and thereby more silver accumulation within the brain. In contrast to the 28-day inhalation study, Sung *et al*. (2009) found a clear dose-dependent increase in the blood silver nanoparticle concentration, indicating a systemic distribution of silver nanoparticles by circulating blood. Finally, based on their observation, these authors estimated an inhalation no observable adverse effect level (NOAEL) of 100 $\mu g/m^3$.

This study by Sung *et al*. has added significantly to advance the knowledge of systemic burden of silver nanoparticles and presents the first chronic inhalation exposure to silver particles, and a start to provide benchmark data for human risk assessment estimates. Sung *et al*. provide key dosing considerations of nanomaterial preparation where their techniques were performed and standardized for direct whole-body animal dosing. Several considerations on nanoparticle stabilities and physical characteristics such as particle temperature when deposed after thermal generation, particle aggregation/agglomeration rates, and surface activities remain unknown. These issues are difficult to assess for their importance, and assumptions must be realized regarding stability of these physical parameters and changes cause dynamic dosing effects. Also following dosing, estimated levels of oral dosing from grooming activities and lung-tracheal-gastrointestinal dose transfer require assessment to their systemic contribution. More importantly, there remains a need to differentiate between soluble/ionic forms of silver in tissues or a test for the presence of insoluble salts, particularly involving the differential accumulation value between the different sexes for liver and kidney burdens. Thus, although this study provides a deep data set and a realistic whole-body exposure paradigm, the distribution of silver nanoparticle after inhalation body burden will require much more information to delineate distribution kinetics/dynamics, toxicity processes, and define key mechanisms for the effects observed following distribution. Many obviously known data requirements remain lacking for human risk assessment such as current data on workplace air concentrations of silver nanoparticles and their release mechanisms and concentrations available from various consumer products. As with most published work in nanotoxicology, the high concentrations used in this study may be difficult to translate to a realistic human chronic exposure scenario. We applaud the authors for providing this study to begin benchmarking lung dosing with the rat model and their procedures to expose defined newly generated silver metal nanoparticles through inhalation whole-body exposure. This work provides key information on possible adverse effects and a first look toward establishing a human NOAEL. These data are positioned to provide a springboard for other researchers to create further data and provide initial data and knowledge for this important deficiency in this rapidly evolving area of human exposure concern.

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